

WHAT IS CLAIMED IS:

1. A method for recovering a desired target nucleic acid molecule from a sample containing a mixture or library of single-stranded nucleic acid containing said molecule, wherein said method comprises the steps:

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- A. incubating said sample containing said nucleic acid mixture or library in the presence of a primer nucleic acid molecule complementary to a sequence of said desired target molecule; said incubation being under conditions sufficient to permit hybridization between said primer and said desired target molecule, and further sufficient to permit the template-dependent extension of said primer to thereby generate a double-stranded desired target molecule;
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- B. transforming single-stranded and double-stranded members of said mixture or library into a host cell, and
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- C. recovering said desired molecule from said cell.

20 2. The method of claim 1, wherein prior to commencing step A, said method comprises the presteps:

- (1) incubating an initial sample containing said nucleic acid mixture or library in the presence of a haptenylated nucleic acid probe molecule, said probe molecules having a sequence complimentary to a nucleotide sequence of said desired target molecule; said incubation being under conditions sufficient to permit said probe to hybridize to said desired target molecule and to thereby generate a hybridized molecule
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- 30 wherein said target molecule is bound to said probe;

- 5 (2) incubating said sample containing said nucleic acid mixture or library and biotinylated probe-target hybridized molecules of prestep (1) in the presence of a binding ligand of the hapten of said haptenylated probe, said binding ligand being conjugated to support; said incubation being sufficient to permit said probe molecules, and said probe-target hybridized molecule to become bound to said binding ligand of said support;
- 10 (3) recovering said probe-target hybridized molecules bound to said support from said nucleic acid mixture or library and any unbound biotinylated probe-target hybridized molecules of prestep (2); and
- 15 (4) incubating said recovered support containing said bound probe-target hybridized molecules under conditions sufficient to separate the strands of double-stranded molecules; said incubation thereby releasing said hybridized target molecule from said biotinylated probe, and generating a sample single-stranded desired target molecule for use in step (A).
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25 3. The method of claim 1, wherein said single-stranded nucleic acid molecule of said sample contains a nucleotide analog, and wherein after completing step A, but prior to commencing step B, said method additionally comprises the presteps:

- 30 (1') incubating said generated double-stranded molecules in the presence of a nuclease capable of degrading nucleic acid containing nucleotide analog residues; and

(2') incubating non-degraded nucleic acid with a primer under conditions sufficient to permit said primer to be extended in a template-dependent manner.

5 4. The method of claim 1, wherein in step A, said template-dependent extension of said primer is conducted in the presence of a nuclease resistant nucleotide analog to thereby generate a double-stranded desired target molecule containing a residue of said nucleotide analog;
10 and wherein prior to commencing said step B, said method additionally comprises the presteps:

15 (1") incubating said generated double-stranded desired target molecule in the presence of a nuclease, wherein said nuclease is substantially unable to cleave a nucleic acid molecule containing said nucleotide analog residue, but is substantially capable of degrading both
20 single-stranded nucleic acid molecules and double-stranded nucleic acid molecules that lack said nucleic acid analog residue; said incubation being under conditions sufficient to permit such degradation, and thereby substantially eliminating both single-stranded
25 nucleic acid molecules and double-stranded nucleic acid molecules that lack said nucleic acid analog residue from said sample; and thereby forming a preparation having a substantial enrichment of said desired target molecule relative to said initial sample; and
30 (2") recovering said desired molecule from said preparation of prestep (1") to thereby form a library or mixture for said step B.

2 5. The method of claim 1, wherein in step A said ~~desired~~ incubation is under conditions which minimize random hybridization.

5 6. The method of claim 2, wherein in prestep (1) said desired incubation is under conditions which minimize random hybridization.

7. The method of claim 1, wherein said desired target nucleic acid molecule is a DNA molecule.

10 8. The method of claim 7, wherein said DNA molecule is a single-stranded DNA molecule.

9. The method of claim 1, wherein said desired target nucleic acid molecule is an RNA molecule.

15 10. The method of claim 1, wherein said desired target nucleic acid molecule is a single-stranded nucleic acid molecule.

11. The method of claim 1, wherein said desired target molecule is a circular nucleic acid molecule.

12. The method of claim 11, wherein said desired target molecule is a circular DNA molecule.

20 13. The method of claim 2, wherein said hapten is biotin, and wherein said binding ligand of said hapten is avidin, streptavidin, or an antibody or antibody fragment that binds biotin.

25 14. The method of claim 13, wherein said ^{BIOTIN-}binding ligand of ~~biotin~~ is avidin.

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15. The method of claim 13, wherein said binding ligand of biotin is streptavidin.

16. The method of claim 2, wherein said support of said prestep (2) is a paramagnetic bead.

5 17. The method of claim 16, wherein said ~~haptenylated probe-target~~ hybridized molecule bound to said paramagnetic bead is recovered by magnetic means.

10 18. The method of claim 2, wherein in said primer molecule of step A is complementary to the same sequence of said desired target molecule as said probe molecule of substep (1).

15 19. The method of claim 2, wherein in said primer molecule of step A is complementary to a sequence of said desired target molecule that differs from the sequence of said desired target molecule that is complementary to said probe molecule of substep (1).

20 20. The method of claim 3, wherein said nucleic acid analog is deoxyuridine, and wherein said nuclease is UDG.

20 21. The method of claim 4, wherein said nuclease does not cleave hemimethylated DNA.

22. The method of claim 21, wherein said nucleic acid analog is 5-methylcytidine, and wherein said nuclease that does not cleave hemimethylated DNA is HhaI.

25 23. The method of claim 2, wherein in prestep (1), said probe has a degenerate sequence.

24. The method of claim 4, wherein in step A, said primer has a degenerate sequence.

25. The method of claim 1, wherein said host cell is a bacterium.

5 26. The method of claim 1, wherein said method additionally includes the step of amplifying said desired target molecule by an in vitro amplification reaction.

27. The method of claim 26, wherein said in vitro amplification reaction is a polymerase chain reaction.